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ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2019, 8(3): 53-58

Chemometrics Assisted Raman Spectroscopy: A Non-Invasive Approach in Diagnosis and Monitoring of Colorectal Cancer

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ABSTRACT

Objective: *Colorectal cancer is a commonly encountered cancer worldwide about 1.4 million new cases diagnosed and 693,900 deaths occurred per year. Colorectal cancer could be stopped and highly curable if diagnosed early. In this proposed study, the main goal is to develop accurate, sensitive and rapid Raman spectroscopy method in colorectal cancer diagnosis of formalin-fixed paraffin embedded tissue samples.* ***Methods:*** *In the proposed method, samples were deparaffinized and prepared as 20 microns of dimension that was located on a coverslip. The instrument produces a continuum laser at 785 nm that were applied to both healthy and cancer tissues. Wavenumber of 50-1800 cm^{-1} was scanned to get information about the metabolic variation in each group.* ***Results:*** *Accuracy of the method was calculated by comparing the results regarding the histopathological evaluation. Healthy and cancer tissues formed two unique clusters via chemometrics algorithm. The rapid, easy and precise diagnosis was achieved for colorectal cancer diagnosis. By this method, some beneficial information regarding the variation in several metabolites was also obtained from the spectrum.* ***Conclusion:*** *It is reported that the optimized method represents an important opportunity for clustering and separating cancer tissues from healthy ones. This novel, rapid, precise and numerical approach may be an effective alternative for the conventional methods.*

Keywords: Colorectal cancer, Raman spectroscopy, Chemometrics, Raman mapping, Qualitative analysis

INTRODUCTION

Cancer takes place in a huge amount of disease, influencing almost 90.5 million people worldwide in 2015 [1]. Colorectal cancer could be formed in both colon and rectum. The term colorectal cancer is defined to explain the uncontrolled cell reproduction in either rectum or colon. Colorectal cancer, commonly encountered cancer worldwide about 1.4 million new cases diagnosed and 693,900 deaths occurred per year [2-4]. Early diagnosis is still the most effective strategy to prevent death [5]. Colorectal cancer is curable during the early stages [1,6-9]. Most common early-diagnosis methods were reported as colonoscopy and digital rectal examination [10,11]. Mortality rates were dramatically reduced in early stage diagnosis [4,12]. Improvements in early cancer diagnosis play a crucial role in reducing the mortality rate [13]. In recent days, histopathological tests are still accepted as the most confident method in diagnosis and photonics strategies like FT-IR and Raman spectroscopy methods were also improved by the help of chemometrics [5]. These invasive methods have several advantages as collecting information from the tissue and give an objective decision as cancer positive and negative. Raman spectroscopy researches are frequently improved in cancer studies [13,14]. Raman spectroscopy has been mostly applied in due to its potential *in vivo* diagnostic tool that can provide data regarding both the chemical and morphologic contents of tissue and discrimination of healthy and cancer tissue [1,13,15,16]. Raman spectroscopy could also be used in the molecular characterization of biocompounds [17,18]. Primarily, Raman spectroscopy needs the illumination of a biological aliquot with a monochromatic laser and subsequent collection and evaluation of the scattered light to get information about the alteration in intensity and wavelength. Raman spectroscopy has several advantages as rapidity, reproducibility, without staining, non-destructive feature, which when combined with effective statistical approaches permits objective discrimination [5,13]. However, histopathological evaluation is postponed to several days to form pathology report via tissue fixation, sectioning and

staining have related costs and manpower [5]. In addition to this, the consumption of several hazardous and expensive chemicals is another disadvantage of histopathological tests. Raman spectroscopy recommends an alternative way promising minimum usage of time, manpower and chemicals. Assisted with chemometrics techniques, Raman spectroscopy presents a more objective diagnostic approach including numerical results [5]. In literature, there are several studies dealing with a colorectal cancer diagnosis in human and mice by Raman spectroscopy method [10,19-21]. In this proposed method it is aimed to develop a new alternative chemometrics assisted method to separate and characterize colon cancer tumors from healthy cells by simple, cheap and rapid Raman spectroscopic method with good accuracy and sensitivity from formalin-fixed paraffin embedded human tissue samples. Secondly, it is aimed to make a tissue imaging of each group benefited from Raman spectroscopy to see differences in collagen bands.

MATERIALS AND METHODS

Analysis of Raman was carried out in backscattering geometry via a WItech Alpha 300R Confocal Raman microscope equipped with a Raman Spectroscopy System UHTS 300 charge coupled with the device operating at 60°. The grating was rearranged at 600 g/mm while BLZ at 750 nm. Vertical shift speed was set at 16.25 µs and horizontal shift speed was 0.033 MHz. All samples were exposured to 785 nm of the laser as excitation sources. This wavelength was found to be optimum for tissue analysis by discarding strong fluorescence emissions that lead to interference for desired signals. Raman signals were received through a confocal pinhole which is 100 µm in diameter via using a 0.9 numerical aperture (NA) objective of 100X magnification. Rayleigh scattering, fluorescence emission, humidity and dust interferences were discarded by this software. Total 22 different subjects were kindly taken from Department of Pathology, Faculty of Medicine, Ataturk University (human ethics committee number: B.30.2.ATA.0.01.00/146). Histopathological diagnosis test was exerted into 22 different tissue samples as a reference method to control the accuracy of the proposed method. The reference method requires almost 3 days of analysis time. According to the reference method, colorectal cancer was detected for 10 samples and 12 samples were found as healthy and did not exhibit any cancerous feature. All samples were deparaffinized and carefully sliced (1 cm × 1 cm). The thickness of each sample was about 20 µm. After prepearing each sample, they were put onto a coverslip and 785 nm laser was applied to get Raman spectra. Data were obtained as ASCII format and exported to MATLAB PLS toolbox 8.0 for chemometrics analysis. Raman mapping was also performed by utilizing two-laser beams by filtering the Rayleigh scattering. Sample spectrum may contain high mounts of interferences in solid samples due to the basic handicaps of Raman spectroscopy; light scattering, the difference in spectroscopic path length variation of the sample and homogenuosity problems especially in biological samples. In order to avoid such unexpected systematical variation, 2 different models were applied for pre-processing the raw data which are differentiation and signal correction. Savitzky-Golay smoothing, Multiple signal correction (MSC), fourier transformation, orthogonal signal correction (OSC) are the most common algorithm on pre-processing of raw spectra. These signal correction algorithms are principled a mathematical function which filters the results of any spectroscopic data to remove unexpected light scattering interferences to stimulate the sensitivty and selectivity of the method. In this study, it is aimed to separate healthy and cancer tissues into 2 main clusters. In order to do this, OSC pre-processed Orthogonal partial least square (OPLS) algorithm were applied to raw Raman spectrometry data.

Generally, the PLS algorithm was fomulated as folow:

$$X = TP^T + E = \sum t_f p_f^1 + E$$

$$X = UQ^T + F = \sum u_f q_f^1 + F$$

where T and U are the score matrices while P and Q are the loading matrices. Residual matrices are referred to as E and F, respectively. In this procedure, OSC is carried out to remove scores of undesired interferences by protecting the needed information. This filter lowered the complexity of the multivariate model. OSC is used as a pre-processing point before the latent variable determination. The general formula of the OSC model is monitored as:

$$X = t_{osc} p_{osc}^T + X^1$$

In this formula, single OSC components are referred to as t_{osc} and p_{osc}. X^1 is the OSC-filtered matrix. By this algorithm, raw Raman spectra data was filtered and reduced with relatively high sensitivity and specificity.

Raman Mapping

Raman map was plotted to utilize WItech Alpha 300R Confocal Raman Microscope. Smooth scan feature was applied to the data. Both scan width and height were set to be 20 µm. Pixels for width and height were arranged to 1024 × 128 that shows an acceptable resolution for both cancer and healthy tissues. Each line 20 points were determined and 50 images were received for each line. Raman mapping was performed between 1200-1700 cm^{-1} wavenumber for monitoring collagen, lipids and amide groups (I, III) formation in cancerous and healthy tissues. Raman mapping images for normal and cancerous tissues were given in Figures 1 and 2.

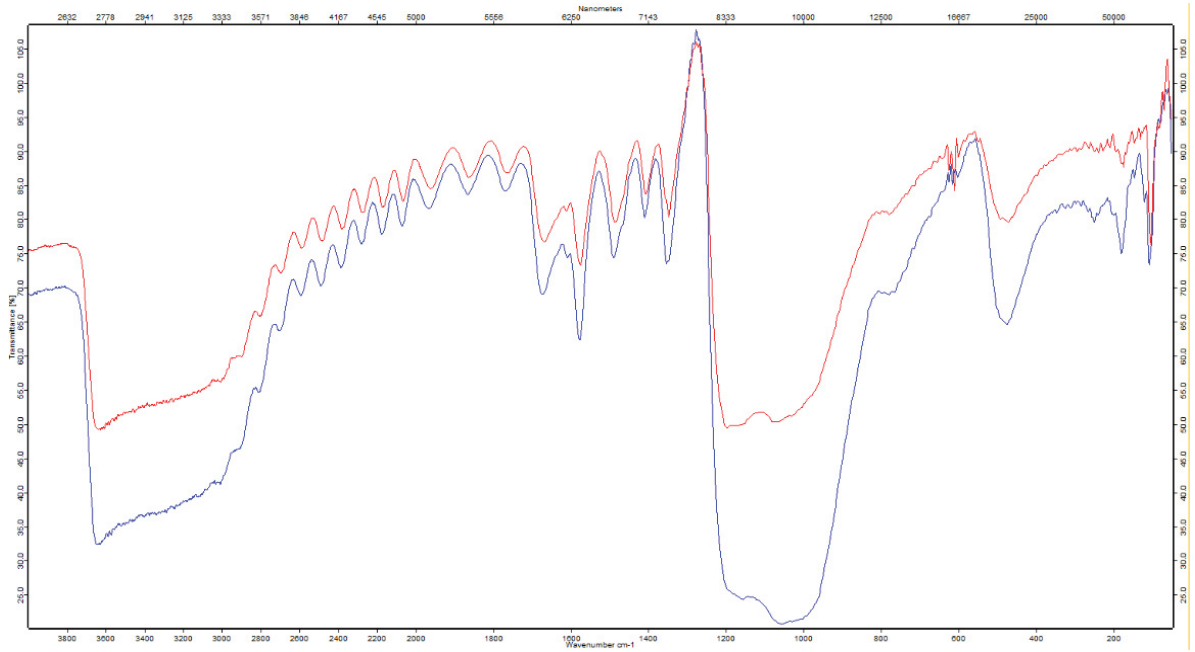

Figure 1 FT-IR spectra of IDC and healthy tissues: differences in the intensity of peaks in the spectra attributed to the chemical compositional and structure changes between IDC and healthy tissues

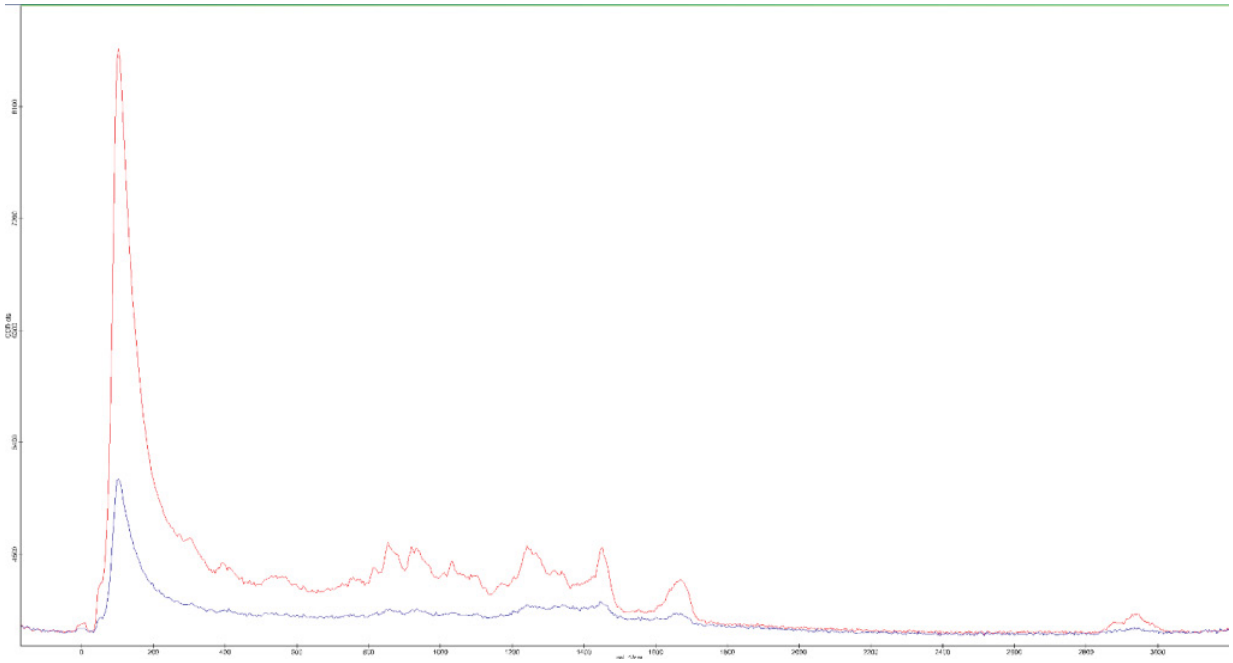

Figure 2 Raman spectra of IDC (blue) and healthy (red) tissues: differences in the intensity of peaks in the spectra attributed to the chemical compositional and structure changes between IDC and healthy tissues

RESULTS

A sample for healthy and cancer tissue Raman spectra was shown in Figure 3. According to the spectra, some important biomolecules were identified. Tyrosine band is encountered at 1209 cm^{-1}, phospholipids were detected at 1130 cm^{-1} [22]. Spectral differences between cancer and healthy tissues were found for 1340 cm^{-1} collogen, 1447 cm^{-1} collogen, lipids, 1275 cm^{-1} Amid III and 1634 cm^{-1} Amid I in agreement with the literature [10,19,23-25]. Collagen, lipids and amide groups (I, III) levels of cancer and healthy tissue were also monitored by Raman mapping to show deformation due to the malignant cells in Figure 3.

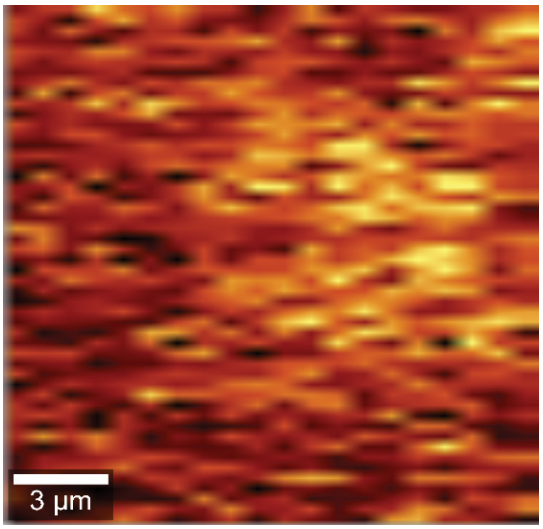

Figure 3 Raman image of healthy tissue

This Raman map shows the differences between collagen, lipids and amide grups (I, III) levels in both tissue samples. Differences are evidently observable between cancer and healthy tissue. Data were also evaluated via chemometrics algorithm. In OSC pre-processed OPLS-DA evaluation, 6 Latent variables were determined to explain the regression analysis. Cross validation of the proposed method was carried out as venetian blinds w/10 splits and 1 sample per split. The statistical parameters indicated that the Root Mean Square Error of Cross Validation (RMSEC) value was found to be 0.102 which is reasonably low. Pareto scaling was used for pre-processing the sample. Each variable was scaled via square root of their standard deviation. A score of latent variables was exhibited in Figure 4. Two different clusters were clearly obtained by this algorithm. Proposed model explain 90.46% of X block. The bias of the model was calculated to be 0.04 (Figure 5).

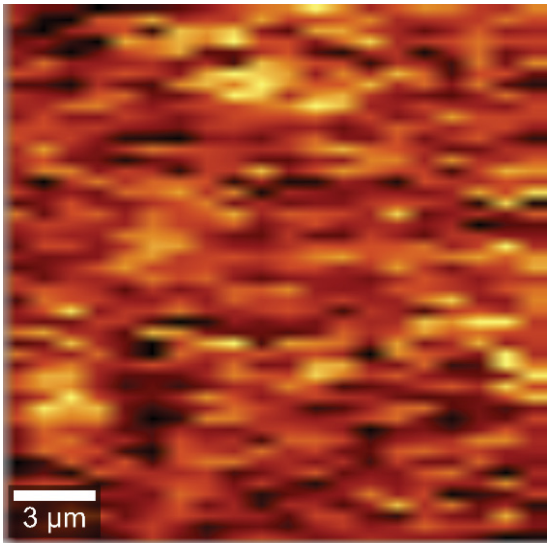

Figure 4 Raman image of cancer tissue

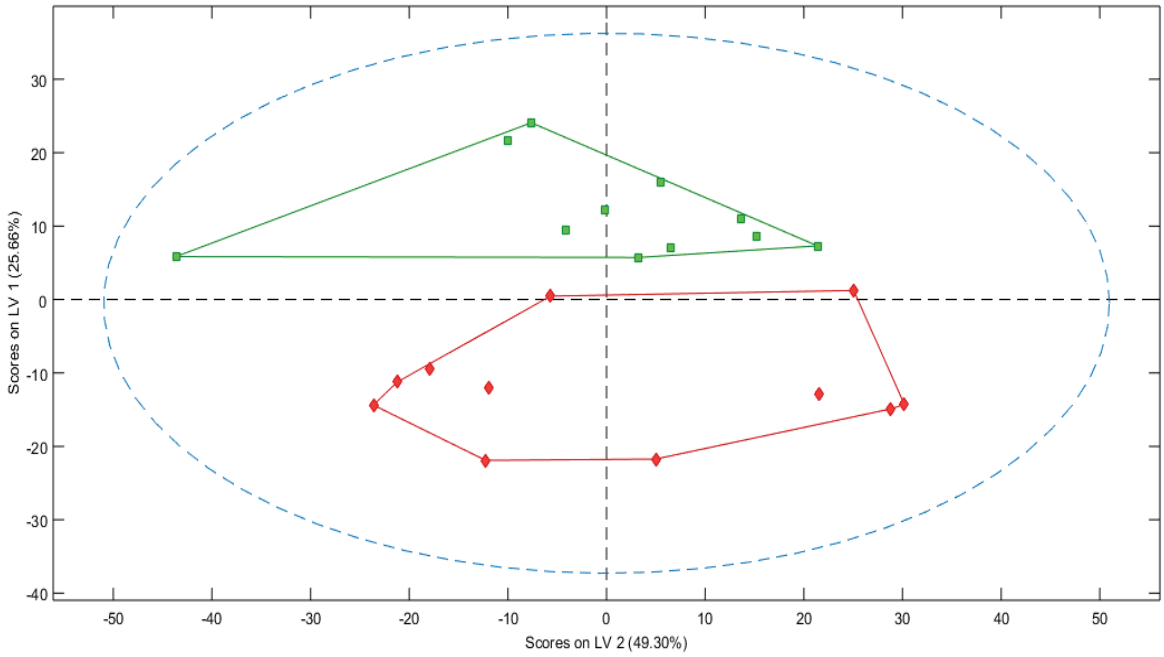

Figure 5 Scatter plot of the latent variable for healthy (green) and IDC (red) breast tissues regarding the OPLS-DA model

CONCLUSION

Colorectal cancer is one of the most encountered diseases in the gastrointestinal system and early, trustworthy diagnosis is crucial. Due to that reason, several alternative methods were developed. In the diagnosis of colorectal cancer, histopathological methods were mostly applied. However, histopathological methods have some limitations and drawbacks which are long run time, subjective results, hazardous chemical usage, and expensive diagnosis kits. Therefore, several alternative methods were tried to be developed by scientists. Proposed method presents a new alternative algorithm for separating cancer and healthy tissue samples via OSC preprocessed OPLS algorithm. Raman spectroscopy technique gives the opportunity to observe differences between cancer tissue and healthy tissue with short run time (approximately 5 min), neither need any chemicals nor expensive diagnosis kit. This method had a perfect correlation with reference histopathological measurements. Furthermore, Raman spectroscopy measurements have numerical results and get more accurate, precise and numeric results. Recovery of the proposed method also supports this claim. So, this technique could be used as an alternative to conventional histopathological methods for diagnosis of colorectal cancer.

DECLARATIONS

Acknowledgement

The authors are grateful for the support Pathology of Department, Faculty of Medicine, Ataturk University and Eastern Anatolia High Technology Application and Research Center, Ataturk University.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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